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X-RAY STUDY OF 1-TOSYL-2- ETHYL PYRROLIDINE TO DETERMINE ITS INCLINATION ANGLE μ_N, SHIFT IN SCREEN SETTING S_N, ABSORPTION COEFFICIENT μ AND SPACE GROUP

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ABSTRACT

Alkaloids usually have complex structure with nitrogen atom involved in the heterocyclic ring. At present more than two thousand alkaloids are known. Broadly alkaloids have been classified into various groups namely Pyrrolizidines alkaloids, Pthalideisoquinoline alkaloids and Pyrrolidine alkaloids. Among these the pyrrolidine alkaloids have been undertaken in the laboratory of department of physics in Jammu University for their crystal analysis. The crystal analysis of 1-TOSYL-2- ETHYL PYRROLIDINE alkaloids was done using oscillation method, Rotation Method and Weissenberg Method. By using all these methods cell parameters like Equi-inclination Angle (μn), Shift in screen setting (Sn) of Weissenberg photograph, linear absorption coefficient (μ) and Space Group were calculated in this study using X-ray diffraction techniques. Density of the compound was determined by floatation technique. The values of μn corresponding to layer line Number 1, 2 and 3 of Weissenberg photograph were found to be 2.984, 5.972 and 8.967 respectively and Sn for the same layer lines were 1.264mm, 2.538mm and 3.826mm respectively. The value of μ and Space Group were found to be 20.941cm-1 and P21212, P212121 respectively. Density of the compound under investigation was found out to be 1.314 gm/cc and was in close agreement with observed density which was 1.309 gm/cc. It was concluded in the research that Unit cells of Pyrrolidine Alkaloids belong to ORTHORHOMBIC crystal system.

KEY WORDS: ORTHORHOMBIC, 1-TOSYL-2-ETHYLPYRR0LIDINE, Weissenberg photograph, Linear Absorption Coefficient, Inclination Angle, Space Group, Pyrrolidine Alkaloids.

INTRODUCTION

Alkaloids usually have complex structure with nitrogen atom involved in the heterocyclic ring (Guha et.al 1979 and Clark et.al, 1970). At present more than two thousand alkaloids are known (Clark et.al, 1970). Carbon, Hydrogen oxygen and nitrogen are the most common constituents of alkaloids (James and Wil-liams, 1972). Alkaloids have been classified on the basis of its chemical, pharmacological and botanical properties. Besides this Alkaloids are classified into various groups namely Pyrrolizidines alkaloids, Pthalideisoquinoline alkaloids and Pyrrolidine alkaloids. Pyrrolizidine alkaloids (PAs) are also called as necine bases. They are a group of naturally occurring alkaloids (Hutchinson et.al, 1974). Pyrrolizidine alkaloids are produced by plants as a defence mechanism against insect herbivores. Soquino Pthalideiline alkaloids are classified into two groups namely classical Pthalideisoquinoline and secopthalideisoquinolines (Simonyi, 1987). Pyrrolidine alkaloids Pyrrolidine, also known as tetrahydropyrrole, is an organic compound with the molecular formula (CH2)4NH. It is a cyclic secondary amine, also classified as a saturated heterocyclic. It is a colourless liquid that is miscible with water and most organic solvents. Pyrrolidine is present in bread, milk, cheese, carrot, and coffee, fatty fish, in the leaves of tobacco, carrot and pharmaceutical drugs (Windholz et.al. 1976). Pyrrolidine is a clear colourless liquid with unpleasant odour or ammonia like odour (Furia, 1973). Several pyrrolidine have been studied, A study done by (Marti, and Carreira, 2003) found that The Spiro (pyrrolidine-3,3′-oxzdole ring system also possess significant biological activity and are interesting, challenging targets for chemical synthesis) the synthesis of a potent and crystallized human cytosolic phospholipase A2α inhibitor, pyrrophenone (6) which inhibits the isolated enzyme with an IC50 value of 4.2 nM. It was found that Pyrrophenone shows potent inhibition of arachidonic acid release, prostaglandin E2, thromboxane B2, and leukotriene B4 formation in human whole blood. The magnitudes of prostaglandin E2 and thromboxane B2 inhibition are the same as those of indomethacin (Seno et.al, 2001). Synthesis of 1-TOSYL-2-ETHYL pyrrolidine compound has already been studied in which preliminary X-ray study was carried out in which the alkaloid was extracted by the reduction of L-pyrrolidine with LAH (Lithium aluminium hydride) and THF (Trihydrofuran) by stirring and cooling resulting in the formation of L-Pyrolinole which is then added to P-Toluine sulphochloride giving to the formation of N-Ditosyl-Pyrolinole (Otani and Yamada, 1973). Further N-Ditosyl-Pyrolinole is added in 200 ml dry THF resulting in the formation of 1-Tosyl-2-Ethyl pyrrolidine as shown in figure 1 (reaction mechanism of M2 crystal) (Otani and Yamada, 1973). Finally the compound is purified by column chromatography on adding SiO2 with benzene (Otani and Yamada, 1973). Few pieces of finally purified material were picked up for X-ray examination. The chemical structure of M2 crystal is shown in **figure 2.**

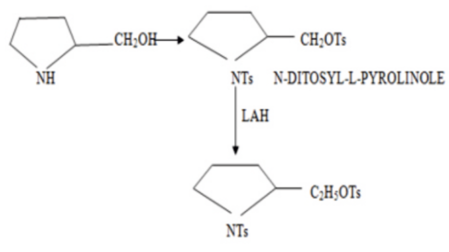

Figure 1: Reaction Mechanism of M_2 Crystal

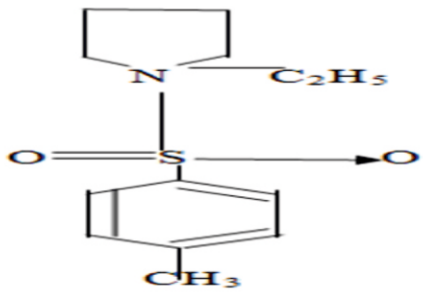

Figure 2: Chemical Structure of titled Crystal

The main aim of the paper was to find out its Equi-inclination Angle (μ_n), Shift in screen setting (S_n) of Weissenberg photograph, linear absorption coefficient (μ) and Space Group of 1-TOSYL-2-ETHYL pyrrolidine using X ray examination which is given the name M_2 by the scientists of Regional research laboratory Jammu. Thus knowing that to which group 1-TOSYL-2-ETHYL pyrrolidine belongs.

MATERIAL AND METHODS

The compound under investigation namely 1-TOSYL-2-ETHYL pyrrolidine has been obtained from chemistry division of RRL Jammu and work was carried out in Jammu University. This work consists of the methods which have been used for the preliminary X-ray study carried out on the compound. A single oscillation photograph has been taken. Since the distance between the layers as obtained from the single oscillation is very small, hence the alignment of the crystal using double oscillation is not possible. A rotation photograph about b-axis is taken and equi-inclination setting parameters have been calculated. The zero-level Weissenberg photograph taken along b-axis has been explained by indexing the photograph with the help of Buerger template. The unit cell parameters were obtained from rotation and zero-level Weissenberg photograph. The upper layer Weissenberg photograph was also taken. The number of molecules per unit cell

was also calculated after measuring the density of the crystal by Flotation method. The crystallographic data obtained from the study helped in finding out the absorption coefficient and space group of the crystal

The 1-TOSYL-2- ETHYL pyrrolidine crystallizes into rectangular shaped crystal. These crystals are then examined under polarizing microscope to ensure that they do not possess any defect like twinning, cracks etc. After the careful examination of all the crystal pieces, a crystal with no defect is chosen.

The selected crystal is mounted properly on goniometer head. One of the axes of crystal is chosen as the axis of rotation. The crystal is stuck to one end of a thin glass fibre, which is chosen because it has minimum scattering and absorption of X-rays. The other end of glass fibre is set within the grove of small spindle on the top of goniometer head with the help of plasticine. In the beginning each arc and sledge of goniometer is set at zero position. The alignment of the crystal is roughly made with the help of microscope fitted with the base of goniometer. One of the prominent faces of the crystal is made parallel to the horizontal cross wire. Then by rotating the crystal through 90^0 and using the sledge and arc, one face of the crystal is again made parallel to the horizontal crosswire. This process is repeated many times till the crystal remain fixed with respect to cross-wire when it is rotated through 360^0. The crystal is thus approximately aligned.

The ultimate evidence of good crystal is normally furnished by diffraction pattern by performing experimental methods.

Oscillation method: This technique is applied only to the preliminary X-ray study of crystals. In this monochromatic X-rays are obtained by use of an appropriate filter placed in the path of X-rays. Practically the oscillation range lies between 10^0 to 20^0 and it can be achieved by the mechanism of two pins provided in the camera arrangement. Due to this arrangement fewer reciprocal lattice points pass through the diffracting position and probability of spot overlap is reduced. Very limited information is obtained from this method. One can establish whether the crystal has mirror plane or not. It can give us information about rotation axis. It is also used for alignment of crystal. There are some difficulties in interpreting and indexing the oscillation photograph. In an oscillation photograph of misaligned crystal, the layer lines become tilted with respect to the central axis of the film. Any error due to mis-setting of the crystal can be thought of as the combination of tilt and bow errors. If the setting error is in the horizontal plane, the trace of layer lines on the film is tilted away from the central line and is called as "bow" error. If the error is in the vertical plane, then the trace of layer lines on the film is then tilted with respect to the central axis of the film and it is called "tilt" error. This experimental method is used to correct these errors.

Correction of tilt and bow error: A double oscillation photograph is taken to correct tilt and bow error. After the first exposure for one hour, the crystal is rotated through 180^0 and film cassette is given a shift of 2mm Exposure to the same film is given for the same range of oscillation but for the difference of time duration of exposure time.

After developing the film, the distance between the two images of zero layer line is measured in millimetres at points 90^0(45mm) to the right ($\Delta r^{'}$) and to the left ($\Delta l^{'}$) of the direct beam points. The difference between these parameters will be positive if the lines have same relative positions as at the centre of film, and it will be negative if they have crossed. The true error is calculated by subtracting the known film shift (Δf) from both the quantities.

$$\Delta r = \Delta r^{'} - \Delta f$$

And $$\Delta l = \Delta l^{'} - \Delta f$$

Tilt error = $\dfrac{\Delta r - \Delta l}{2}$ Bow error = $\dfrac{\Delta r + \Delta l}{2}$

The direction of orientation is determined from the sign of the error but the straight forward approach is achieved by the direct comparison of film and crystal rotation.

Rotation method: This method is rapid and reasonably an accurate method for determining the direct lattice period which coincides with the rotation axis of crystal.

In this method X-rays are generated in X-ray tube and are filtered through a nickel foil of suitable thickness (0.25mm). These X-rays enters a system which permits only a beam of substantially parallel X-rays to reach the crystal. This monochromatic beam is made incident on the crystal which is mounted on the goniometer head and it is centred so that it is at right angle to the direction of X-ray beam. The crystal is made to rotate slowly at a uniform angular rate by a small motor. Then diffracted radiations which are developed when the crystal reaches a certain specific orientations are made to fall on the photographic film. The cylindrical form of film is preferred because it has certain chief advantages over that of flat platform of film mounting such that it is capable of recording a great range of glancing angle θ, and consequently gives a great wealth of reflections. The greater range of glancing angle leads ultimately to the superior accuracy in the refinement of cell constant to the greater wealth of data for the identification of correct space group and finally to the greater accuracy in the determination of position of atoms in the cell.

Actually the rotation method furnishes the shape and size of unit cell of an unknown crystal, provided the crystal can be oriented to rotate about the requisite direction. The rotation photograph furnishes symmetry information indirectly but not from the direct view of the photograph. When the crystal is mounted on the three axes separately and rotation photograph are taken and by the repeat period determined from the photograph, then we can say easily which type of crystal is. Because of this reason, only the rotation method can is considered to be only a supplementary method in the laboratory, where the determination of symmetry is an important part of the problem.

A rotation photograph gives the limited information about the data necessary to identify a particular reciprocal lattice point with a reflection. We can find repeat distance with the help of this method.

Weissenberg method: This method of recording three dimensional X-ray diffracted data on photographic film was introduced by K-Weissenberg in 1924. All crystal structure investigation, no matter how the final intensity data are to be collected, begin with the photographic determination of cell constants and space group. Weissenberg method like rotation method and oscillation method is based upon rotating a single crystal in the beam of X-rays. In this method rotation of crystal is accompanied by the translation of film holder by maintaining a definite instrumental relationship. In Weissenberg instrument, it is customary to have rotation axis of the crystal horizontal, which facilitates the equi-inclination settings by varying μ, the angle between rotation axis and X-ray beam. In this method instead of allowing all Laue cones to record on the single film, one cone at a time is allowed to pass through a gap maintained between two layer line screens. The location of the gap is variable so that any desired Laue cone can be isolated. The distance measured normal to the direction of film translation is proportional to the projection of glancing angle 2θ on a plane normal to the rotation axis.

The proportion is designated by ϒ and its value is provided by proportion given below:

$$\frac{\Upsilon}{X} = \frac{360^0}{2\pi r}$$

$$\Upsilon = 360^0.$$

$$X = \frac{C_1 X}{2\pi r}$$

It is common to give this constant a value of 2^0/mm, and value of r = 28.648mm. The mechanism of Weissenberg instrument causes linear motion of camera travel "Z" to be directly proportional to the crystal rotation "W". The relation is given by

$W = C_2 Z$, Where C_2 is another instrumental constant whose numerical value is 2^0/mm.

Therefore, $C_1 = C_2$. This shows that instrument with undistorted transformation scale records the diffraction data on the film in the form of two dimensional patterns.

RESULTS AND DISCUSSION

Calculation of μ_n and S_n corresponding to different layers of Weissenberg photograph

To record the diffraction data of particular Laue cone, it requires the pre-adjustment of the layer line screens so that the diffracted rays from a single layer can pass through the gap between the screens. There are several methods out of which the equi-inclination setting is the most advantageous. In this case the direct X-ray beam and diffracted beams are equally inclined to the layer of reciprocal lattice, i.e. $\mu_n = \vartheta$, where μ_n is the complement of the angle between the incident beam and the axis of oscillation, while ϑ is the complement of semi-angle of Laue cone.

For equi-inclination arrangement μn is given by

$$\mu n = \sin^{-1}(£n/2)$$

Where £n is the reciprocal distance between the zero and nth layer line and is given by

$$£n = \sin\tan^{-1}(Y_n/R)$$

Where Y_n = distance between nth and zero layer line, and

R = Radius of cylindrical film

The shift S_n towards the crystal to collect nth layer line is given by

$$S_n = r_s \tan\mu_n$$

Where rs is the radius of layer line screen

The value of μn and Sn corresponding to different layers has been calculated as given in Table1. This is used for obtaining higher level Weissenberg photograph

Table 1: Calculation of μ_n and S_n corresponding to different layers of Weissenberg photograph

Radius of cylindrical camera = R = 28.65mm				
Radius of layer line screen = r_s = 24.25mm				
Here the crystal is rotated about b-axis				
Layer line number	**Distance between zero layer and nth layer(Y_n)**	**£$_n$ = sintan^{-1}(Y_n/R)**	**μ_n = sin^{-1}(£$_n$/2)**	**$S_n = r_s \tan\mu_n$**
1	3mm	0.1041mm	2.984	1.264mm
2	6.2mm	0.2082mm	5.974	2.538mm
3	9.4mm	0.3117mm	8.967	3.836mm

Calculation of linear absorption co-efficient

The linear absorption co-efficient of any crystal can be computed from the knowledge of its chemical composition, density and mass absorption co-efficient of the elements. Table of mass absorption co-efficient is given in "international table for X-ray crystallography vol.3". The mass absorption co-efficient are fraction not only of the elements but also of the wavelength of radiation employed.

The linear absorption co-efficient is given by relation

$\mu = G(\mu/\rho)$

Where G is the density of crystal and μ/ρ is the mass absorption co-efficient of crystal. The mass absorption co-efficient for the crystal is computed from the individual mass absorption co-efficient of the elements, A, B, C…………….. in the crystal. Thus the above relation can be represented as

$\mu = GP_A(\mu/\rho) + GP_B(\mu/\rho) + GP_C(\mu/\rho) + \ldots\ldots\ldots\ldots$

Where P_A is the fraction by weight of element A in the compound ABC. Calculation of linear absorption is given in table-2.

Table 2: Calculation of linear absorption co-efficient of the titled compound ($C_{13}H_{19}So_2N$).

Radiation used = cuKα (λ=1.5418*A*)						
Symbol of atom	**Total No. of atom present**	**Atomic weight**	**Total weight of atom specifies in compound**	**Fraction of total weight (P)**	**Mass absorption co-efficient (μ/) In gm^{-1}**	**P(μ/)g^{-1}**
C	13	12	156	0.6160	4.60	2.8336
H	19	1	19	0.0750	0.435	0.0326
S	1	32	32	0.1264	89.1	11.2622
O	2	16	32	0.1264	11.50	1.4536
N	1	14	14	0.0553	7.52	0.4158

From this table,
$\sum P(\mu/\rho) = 15.9978 g^{-1}$

Since density G = 1.309g/cc

Therefore, absorption co-efficient $\mu = 20.941 cm^{-1}$

The calculated value of linear absorption co-efficient for given sample comes out to be 20.941cm^{-1}. This value is large because of presence of sulphur atoms in the given sample. Since the mass absorption co-efficient for sulphur atom is 89.1gm^{-1}, so the linear absorption is large.

Space group determination: The usefulness of Weissenberg photographs in space group determination lies in their ability to show systematic absence or presence or some specific reflections and this imply the presence of certain symmetry element in the crystals and which can be detected by the analysis of reflection data. The indexed data of zero and higher layer Weissenberg photograph taken along b-axis reveal that general conditions for limiting possible reflections for the Orthorhombic space group $P2_12_12$ are satisfied and further that the confirmation of the crystal system has been made on the basis of cell dimension calculations. Based on the above result, the space group for such crystal comes out to be $P2_12_12$ or $P2_12_12_1$ (International Table for X-ray crystallography, vol 1). The reflection data obtained from zero layers and higher layer Weissenberg photograph reveals that the following conditions limiting possible reflections for space group $P2_12_12$ are satisfied.

a) h00: for h = 2n
b) 0k0: for k =2n
c) 00l : No condition

The condition at serial No. c) could be satisfied if the diffracted data along c-axis is taken.

Therefore in order to confirm the existence of space group $P2_12_12_1$, an attempt was made to mount crystal along another axis(c-axis). An oscillation photograph taking c-axis as the axis of rotation was taken wherein layer lines have been found very close to each other and hence was difficult to be resolved. From such a distorted and overlapped photograph, the calculation of Tilt and Bow error and hence the alignment of crystal was found difficult. Hence based on the reflection data of crystal along b-axis and subsequently the cell dimension calculations, it is established that space group for this compound could possibly be $P2_12_12$ though the possibility of the space group $P2_12_12_1$ can also not be ruled out. Table 3 shows the summary of results obtained during the study

Table 3: Results obtained from the preliminary X-ray study of 1-Tosyl-2-Ethyl pyrrolidine

Chemical formula	$C_{13}H_{19}SO_2N$
Radiation used	cuKα (λ=1.5418A^0)
M0lecular Weight	253
No. of molecules per Unit cell	8
Observed density (D_o)	1.314gms/cc
Calculated density (D_c)	1.309gms/cc
Crystal system	Orthorhombic
Space group	$P2_12_12$ or $P2_12_12_1$
Linear absorption co-efficient	20.941cm^{-1}

CONCLUSION

On the basis of experiments in the study following conclusions were drawn

(i) The values of Equi-inclination Angle (μn), corresponding to layer line Number 1, 2 and 3 of Weissenberg photograph were found to be 2.984, 5.972 and 8.967 respectively.

(ii) Shift in screen setting (Sn) for the same layer lines of Weissenberg photograph were found to be were 1.264mm, 2.538mm and 3.826mm respectively

(iii) The value of linear absorption coefficient (μ) was found to be 20.941cm-1.

(iv) Space Group was found to be P21212, P212121 respectively.

(v) Density of the compound under investigation was found out to be 1.314 gm/cc and was in close agreement with observed density which was 1.309 gm/cc.

(vi) The most important revelation of the study was that the compound namely 1-Tosyl-2-Ethyl pyrrolidine belongs Orthorhombic crystal system.

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